COURSE MSc (BIOTECHNOLOGY) III SEM

PAPER CODE: MBT-304

PAPER TITLE: ENZYMOLOGY AND ENZYME TECHNOLOGY

ENZYME INHIBITION

By:

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- Reversible Inhibition- Competitive, Non Competitive, Uncompetitive, Mixed, Substrate, Allosteric and Product Inhibition. Irreversible Inhibition- Suicide inhibition. Mechanism of enzyme action;
- Examples and Mechanism of various Inhibitions like Penicillin, Iodoacetamide and DIPF

ENZYME INHIBITION

Inhibitors are substances which tend to decrease the rate of an enzyme-catalysed Reaction

- Many enzyme inhibitors are effective chemotherapeutic agents, since an "unnatural" substrate analog can block the action of a specific enzyme.
- **For example:**
- Aspirin (acetylsalicylate) inhibits the enzyme that catalyzes the first step in the synthesis of prostaglandins, compounds involved in many processes, including some that produce pain.
- **Methotrexate** (also called **amethopterin**) inhibit enzyme **dihydrofolate reductase,** an essential cofactor in the biosynthesis of the DNA precursor dTMP
- **Methotrexate** is used to treat cancer, psoriasis, rheumatoid arthritis, etc.
- The study of enzyme inhibitors also has provided valuable information about enzyme mechanisms and has helped define some metabolic pathways.

ENZYME INHIBITION

There are two broad classes of enzyme inhibitors: reversible and irreversible.

Reversible inhibitors bind to an enzyme in a reversible fashion and can be removed by dialysis(or simply dilution) to restore full enzymic activity

Reversible inhibitors usually rapidly form an equilibrium system with an enzyme to show a definite degree of inhibition which remains constant over the period

$$E + S \rightleftharpoons [ES] \rightleftharpoons E + P$$

$$E + I \rightleftharpoons [EI] \longrightarrow \text{No reaction}$$

Irreversible inhibitors cannot be removed from an enzyme by dialysis.

The degree of inhibition by irreversible inhibitors may increase over this period of time.

$$E + S \rightleftharpoons [ES] \rightleftharpoons E + P$$

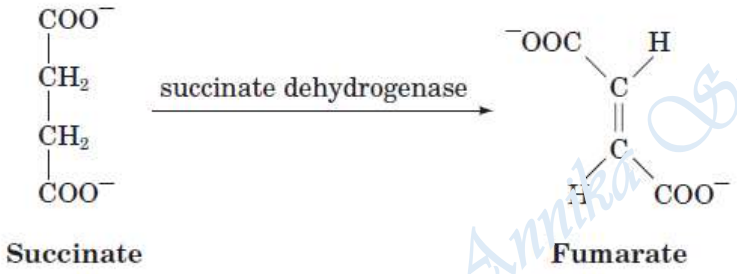
$$E + I \longrightarrow [EI] \longrightarrow \text{No reaction}$$

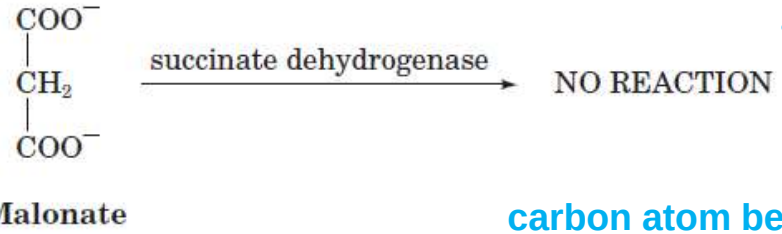
- Types of Reversible Inhibition: (I) Competitive, (II) Non-Competitive, (III) Uncompetitive, and (IV) Mixed Inhibition.

Competitive Inhibition

A substance that competes directly with a normal substrate for an enzymatic binding site is known as a **competitive inhibitor.**

- Such an inhibitor usually resembles the substrate to the extent that it specifically binds to the active site but donot react further.
- **Succinate dehydrogenase,** converts **succinate** to **fumarate**, is competitively inhibited by **malonate**





- **The effectiveness of malonate in inhibiting succinate dehydrogenase suggests that the enzyme's substrate-binding site is designed to bind both of the substrate's carboxylate groups.**
- **Malonate has two carboxyl groups, like the substrate, succinate, and can fill the succinate-binding site on the enzyme.**
- **The subsequent reaction involves the formation of a double bond, and since malonate, unlike succinate, has only one carbon atom between the carboxyl groups, it cannot react.**

Competitive Inhibition

- The general model for competitive inhibition is given by the following reaction scheme:

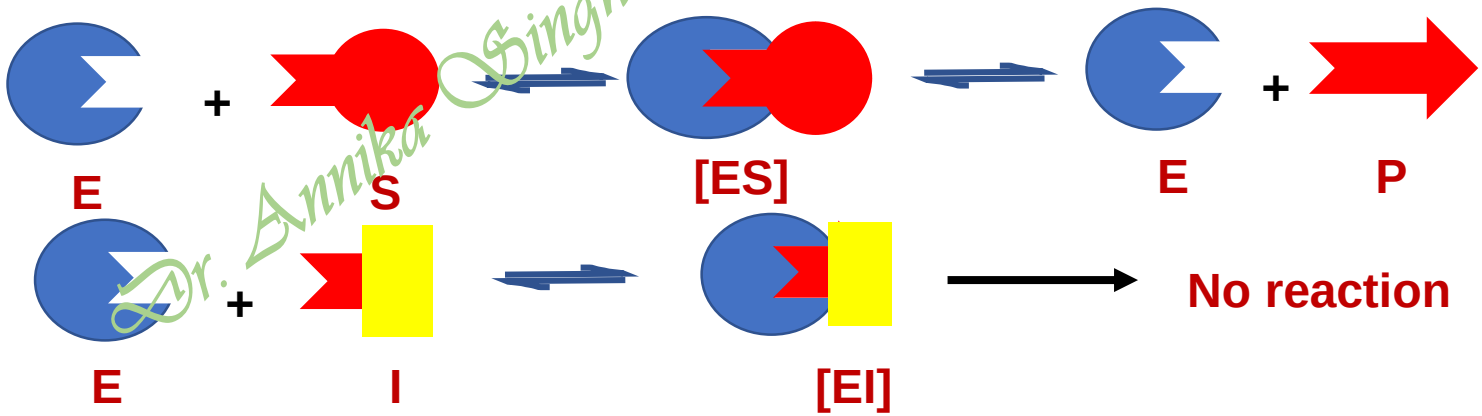
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+$$

$$I$$

$$K_I \rightleftharpoons$$

$$EI + S \longrightarrow \text{NO REACTION}$$



Competitive Inhibition

- it is assumed that I, the inhibitor, binds reversibly to the enzyme and is in rapid equilibrium with it so that
- $$K_I = \frac{[E][I]}{[EI]} \quad (1)$$
- EI, the enzyme–inhibitor complex, is catalytically inactive.
- *A competitive inhibitor therefore acts by reducing the concentration of free enzyme available for substrate binding.*
- For expressing *vo* in terms of $[E]_T$, [S], and [I].
- We must now take into account the existence of EI.
- $$[E]_T = [E] + [EI] + [ES] \quad (2)$$
- The enzyme concentration can be expressed in terms of [ES] by rearranging MM Eq. under the steady-state condition:
- $$[E] = \frac{K_M[ES]}{[S]} \quad (3)$$

Competitive Inhibition

That of the enzyme–inhibitor complex is found by rearranging Eq. [1] and substituting Eq. [3] into it:

$$[\mathrm{EI}] = \frac{[\mathrm{E}][\mathrm{I}]}{K_\mathrm{I}} = \frac{K_M[\mathrm{ES}][\mathrm{I}]}{[\mathrm{S}]K_\mathrm{I}} \qquad (4)$$

Substituting the latter two results into Eq. [14.33] yields

$$[\mathrm{E}]_\mathrm{T} = [\mathrm{ES}]\left\{\frac{K_M}{[\mathrm{S}]}\left(1 + \frac{\mathrm{I}}{K_1}\right) + 1\right\} \qquad (5)$$

which can be solved for [ES] by rearranging it to so that,

$$[\mathrm{ES}] = \frac{[\mathrm{E}]_\mathrm{T}[\mathrm{S}]}{K_M\left(1 + \frac{[\mathrm{I}]}{K_\mathrm{I}}\right) + [\mathrm{S}]} \qquad (6)$$

For Competetive Inhibition

$$[E]_T = E + [EI] + [ES]$$

$$\because [E] = \frac{Km\,[ES]}{[S]}$$

$$\because [EI] = \frac{[E][I]}{K_i} = \frac{Km\,[ES]}{[S]} \cdot \frac{[I]}{K_i}$$

$$\therefore [E]_T = \frac{Km[ES]}{[S]} + \frac{Km[ES]}{[S]} \cdot \frac{[I]}{K_i} + [ES]$$

$$\therefore [E]_T = [ES]\left\{\frac{Km}{[S]}\left(1+\frac{[I]}{K_i}\right)+1\right\}$$

$$[EI] = \frac{[E][I]}{K_I} = \frac{K_M[ES][I]}{[S]K_I}$$

OR $$[ES] = \frac{[E]_T}{\frac{Km}{[S]}\left(1+\frac{I}{K_i}\right)+1}$$

OR $$[ES] = \frac{[E]_T}{\frac{Km\left(1+\frac{I}{K_i}\right)+[S]}{[S]}}$$

OR $$[ES] = \frac{[E]_T[S]}{Km\left(1+\frac{I}{K_i}\right)+[S]}$$

$\because v_o \propto [ES]$ and $V_{max} \propto [E_T]$

$$\therefore \boxed{v_o = \frac{V_{max}[S]}{Km(1+I/K_i)+[S]}}$$

Competitive Inhibition

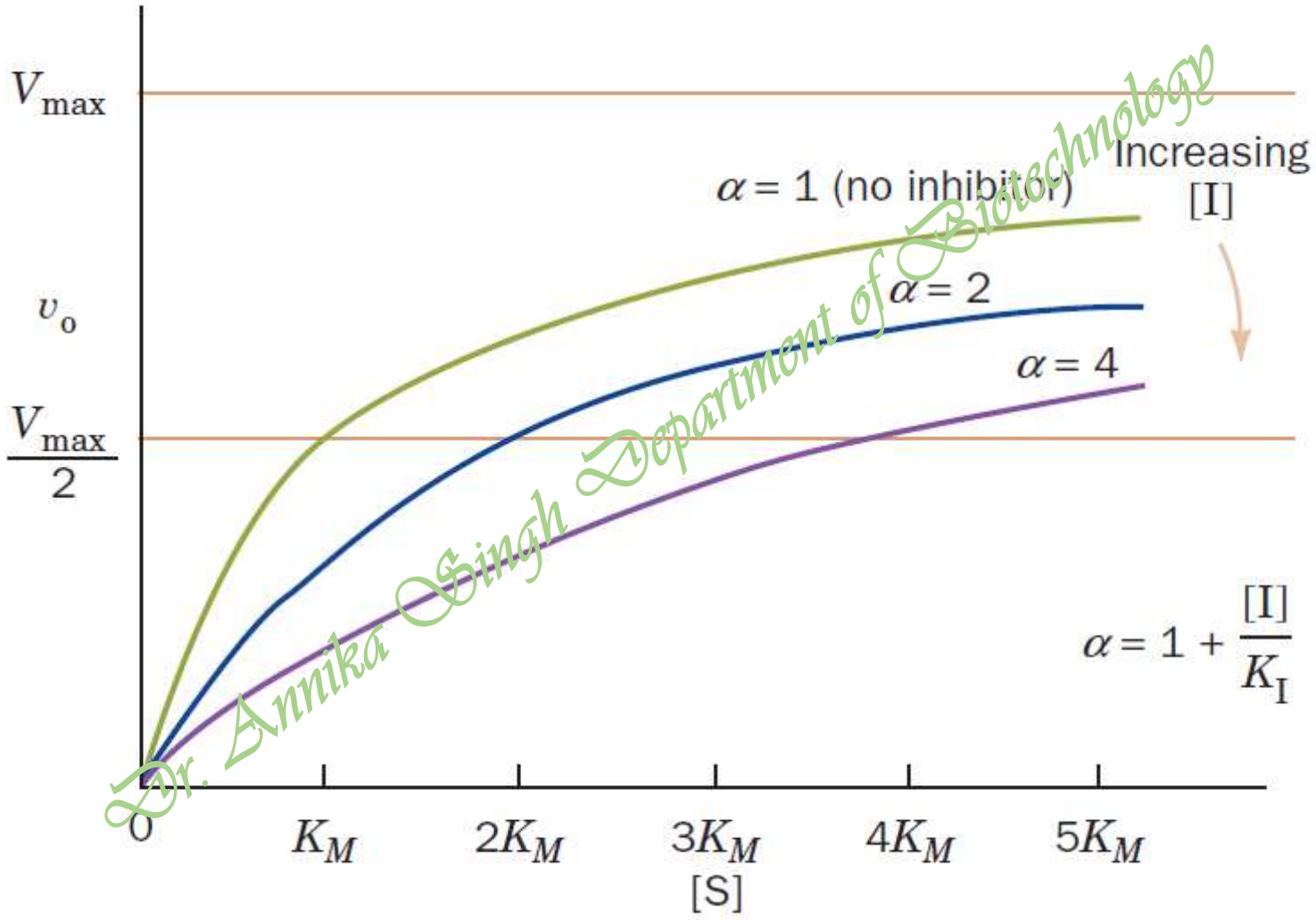
The initial velocity can be expressed as

$$v_o = k_2[ES] = \frac{k_2[E]_T[S]}{K_M\left(1 + \frac{[I]}{K_I}\right) + [S]} \quad (7)$$

If $$\alpha = \left(1 + \frac{[I]}{K_I}\right) \quad (8)$$

$$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]} \quad (9)$$

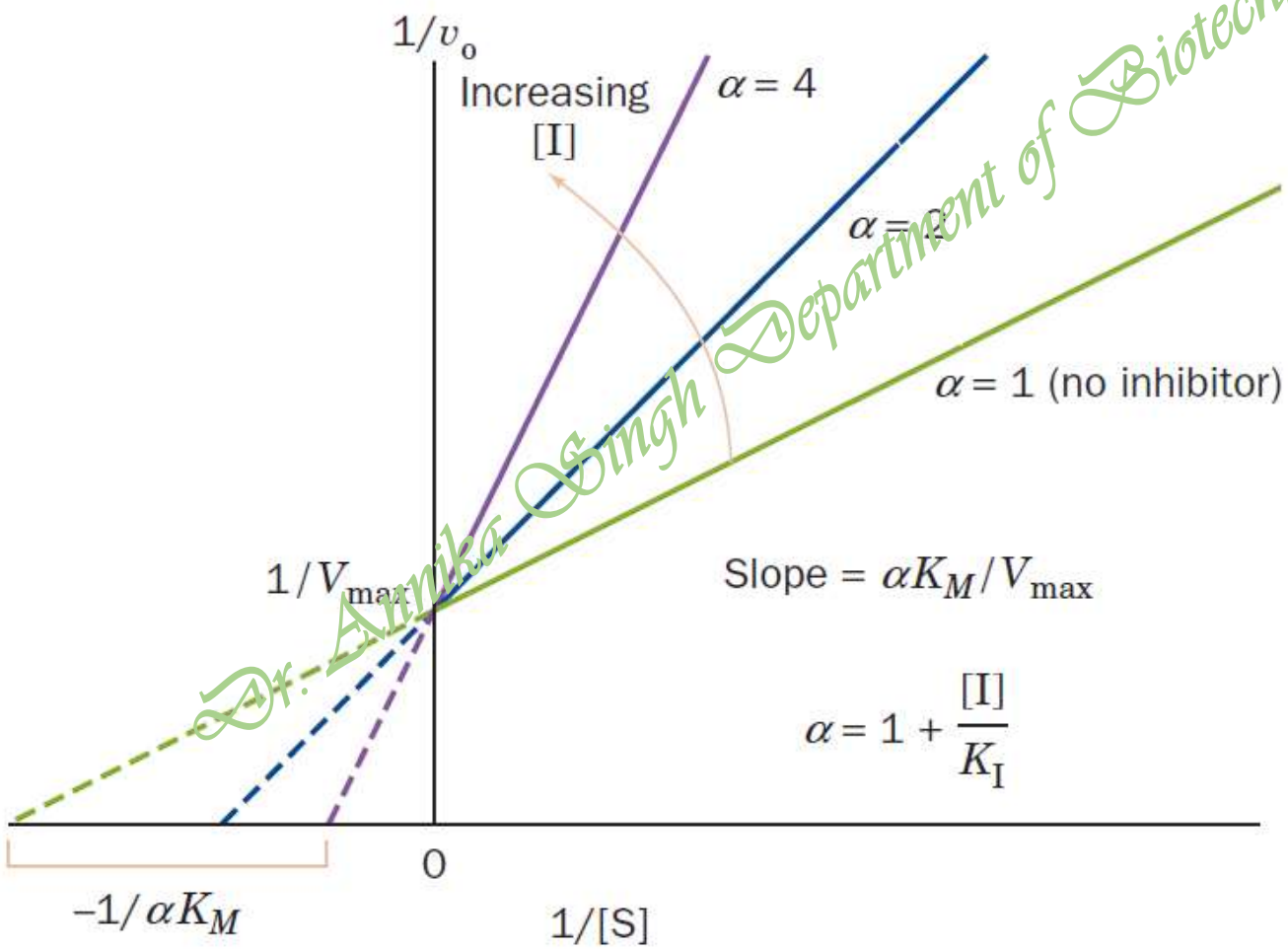
Competitive Inhibition



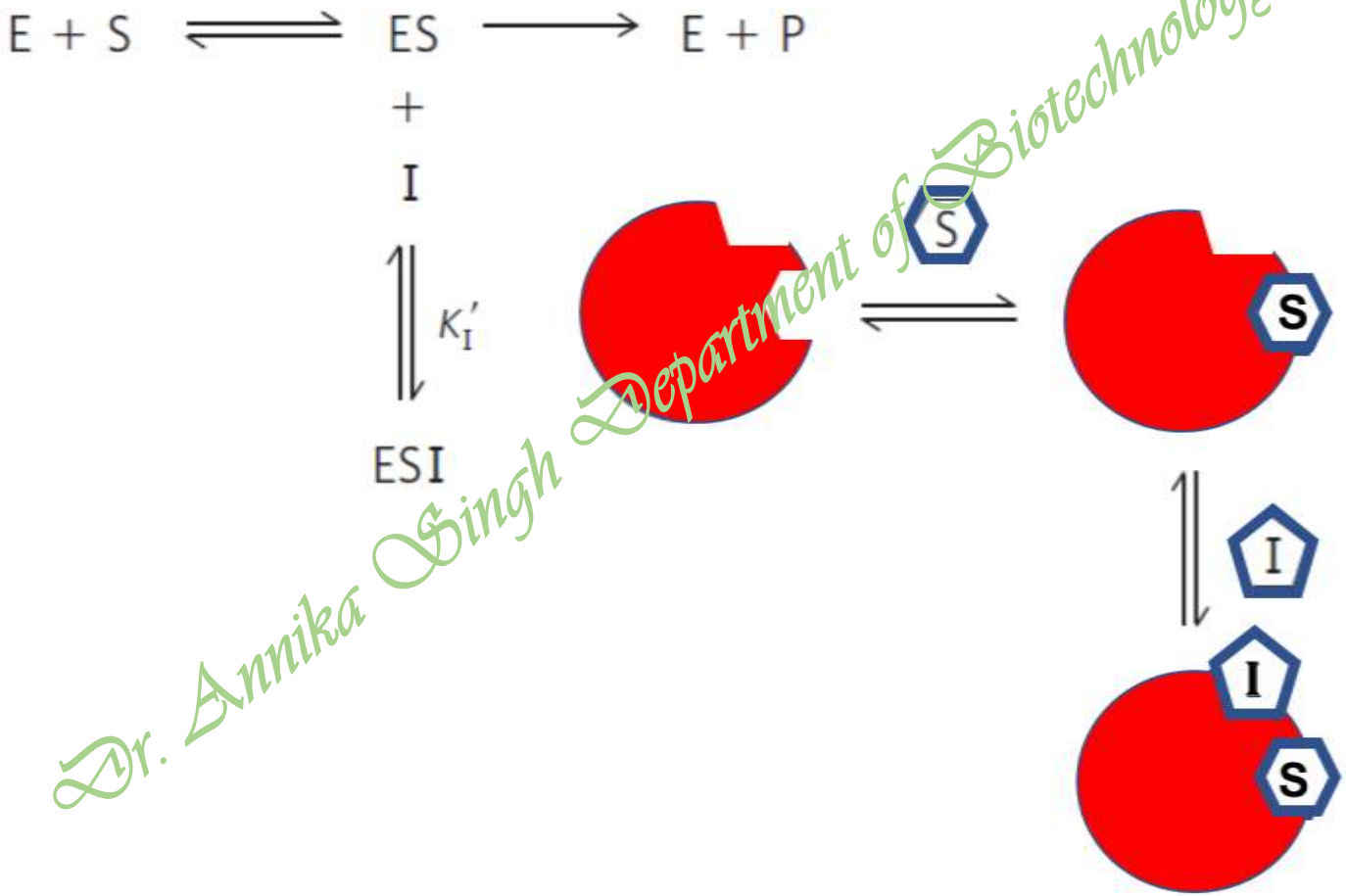
Plot of the initial velocity v_o of a simple M–M reaction versus the substrate concentration [S] in the presence of different concentrations of a competitive inhibitor.

Lineweaver–Burk plot of the competitively inhibited Michaelis–Menten enzyme

$$\frac{1}{v_o} = \left(\frac{\alpha K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

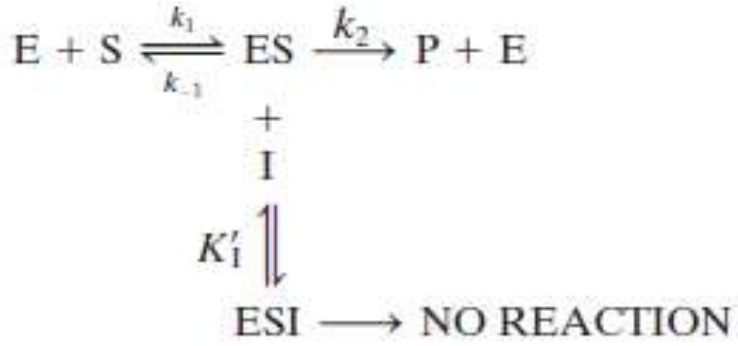


UNCOMPETITIVE INHIBITION



UNCOMPETITIVE INHIBITION

- In **uncompetitive inhibition,** the inhibitor binds directly to the enzyme–substrate complex but not to the free enzyme:



- The inhibitor-binding step, which has the dissociation constant is assumed to be at equilibrium.
- $$K'_I = \frac{[ES][I]}{[ESI]}$$
- The binding of the uncompetitive inhibitor, which need not resemble the substrate, is envisioned to cause structural distortion of the active site, thereby rendering the enzyme catalytically inactive.
- The Michaelis–Menten equation for uncompetitive inhibition,

$$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

UNCOMPETITIVE INHIBITION

For Uncompetitive Inhibition

$$[ES] + [I] \underset{}{\overset{K_i}{\rightleftharpoons}} [ESI] \quad \text{— (1)}$$

$$K_i = \frac{[ES][I]}{[ESI]} \quad \text{— (2)}$$

$$[E]_T = [E] + [ESI] + [ES] \quad \text{— (3)}$$

$$= \frac{K_m [ES]}{[S]} + \frac{[ES][I]}{K_i} + [ES] \quad \text{— (4)}$$

$$= [ES] \left\{ \frac{K_m}{[S]} + \frac{I}{K_i} + 1 \right\} \quad \text{— (5)}$$

UNCOMPETITIVE INHIBITION

$$[ES] = \frac{[E]_T}{\frac{Km}{[S]} + \frac{I}{Ki} + 1} \quad \text{— (6)}$$

OR

$$[ES] = \frac{[E]_T}{\frac{Km}{[S]} + 1 + \frac{I}{Ki}} \quad \text{— (7)}$$

$$\frac{Km}{[S]} + 1 + \frac{I}{Ki} = \frac{Km + [S] + [S]\frac{I}{Ki}}{[S]} \quad \text{— (8)}$$

$$[ES] = \frac{[E]_T [S]}{Km + [S]\left(1 + \frac{I}{Ki}\right)} \quad \text{— (9)}$$

UNCOMPETITIVE INHIBITION

By Substituting v_o for ES & V_{max} for $[E]_T$ equation 9 transform into

$$v_o = \frac{V_{max}[S]}{K_m + [S]\left(1 + \frac{I}{K_i}\right)} \quad \text{— (10)}$$

if equation 10 is devided by $(1 + I/K_i)$

$$v_o = \frac{\frac{V_{max}}{(1 + I/K_i)}[S]}{\frac{K_m}{(1 + I/K_i)} + [S].} \quad \text{— (11)}$$

MM equation 11 for Uncomp. [I]

The conservation condition is

$$[E]_T = [E] + [ES] + [ESI] \quad (1)$$

OR

$$[E]_T = [ES]\left(\frac{K_M}{[S]} + 1 + \frac{[I]}{K_I'}\right) \quad (2)$$

$$[ES] = \frac{[E]_T}{\left(\frac{K_M}{[S]} + 1 + \frac{[I]}{K_I'}\right)}$$

Defining α' as

$$\alpha' = 1 + \frac{[I]}{K_I'} \quad (3)$$

and v_o and V_{max} as $k_2[ES]$ and $k_2[E]_T$, respectively,

$$v_o = k_2[ES] = \frac{V_{max}}{\frac{K_M}{[S]} + \alpha'} \quad (4)$$

UNCOMPETITIVE INHIBITION

- The Michaelis–Menten equation for uncompetitive inhibition,

$$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]}$$

This equation indicates that *at high values of [S], v_o asymptotically approaches* V_{max}/α', *so that, the effects of uncompetitive inhibition on Vmax are not reversed by increasing the substrate concentration.*

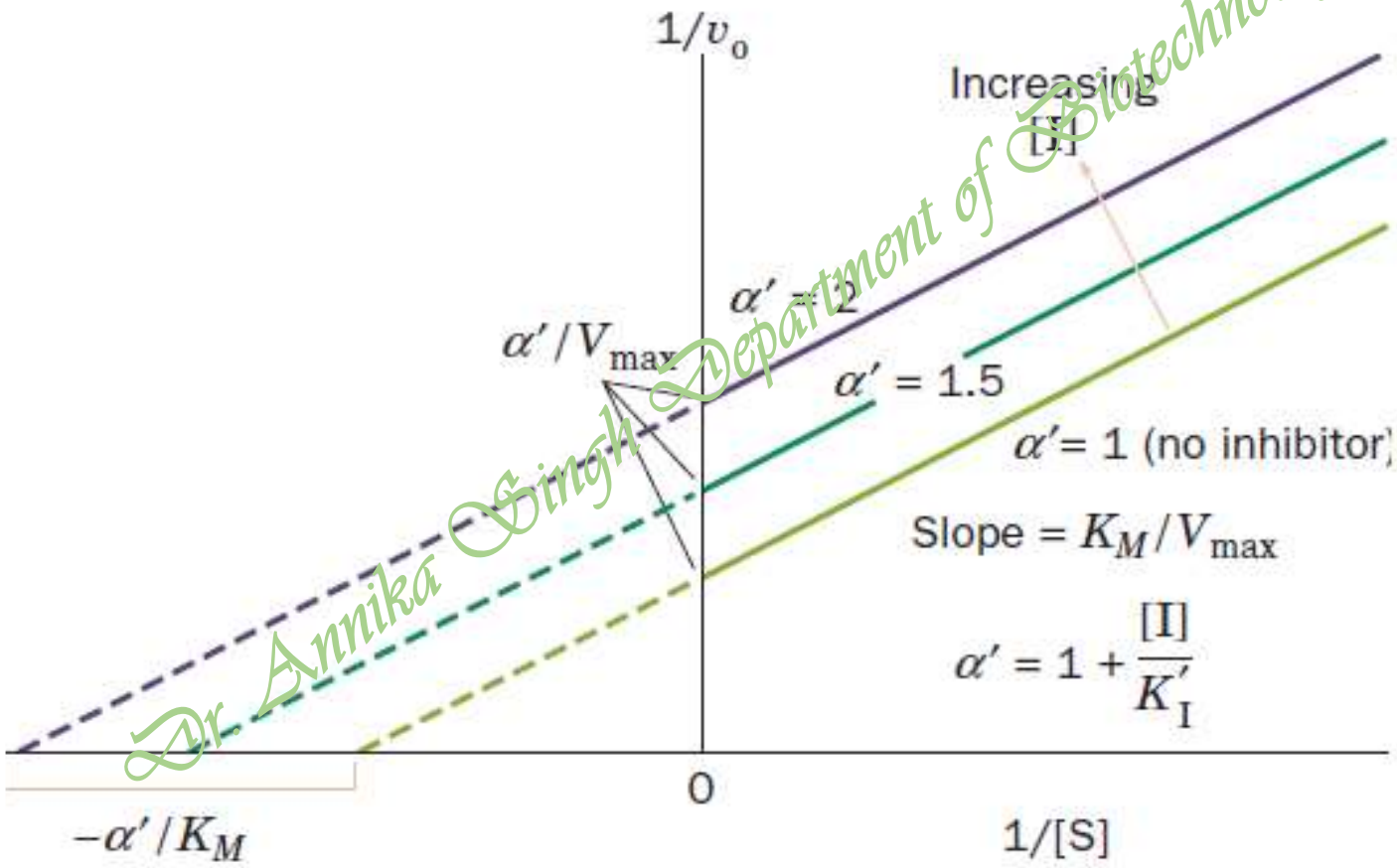
However, at low substrate concentrations, that is, when [S] << K_M, the effect of an uncompetitive inhibitor becomes negligible, again the opposite behavior of a competitive inhibitor. When cast in the double-reciprocal form, becomes

$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$

The Lineweaver–Burk plot for uncompetitive inhibition is linear with slope K_M/Vmax, as in the uninhibited reaction, and with $1/v_o$ and 1/[S] intercepts of α /*V*max and -α'/K_M, respectively.

A series of Lineweaver–Burk plots at various uncompetitive inhibitor concentrations consists of a family of parallel lines This is diagnostic for uncompetitive inhibition.

UNCOMPETITIVE INHIBITION



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- Reversible Inhibition- Competitive, Non Competitive, Uncompetitive, Mixed, Substrate, Allosteric and Product Inhibition. Irreversible Inhibition- Suicide inhibition. Mechanism of enzyme action;
- Examples and Mechanism of various Inhibitions like Penicillin, Iodoacetamide and DIPF

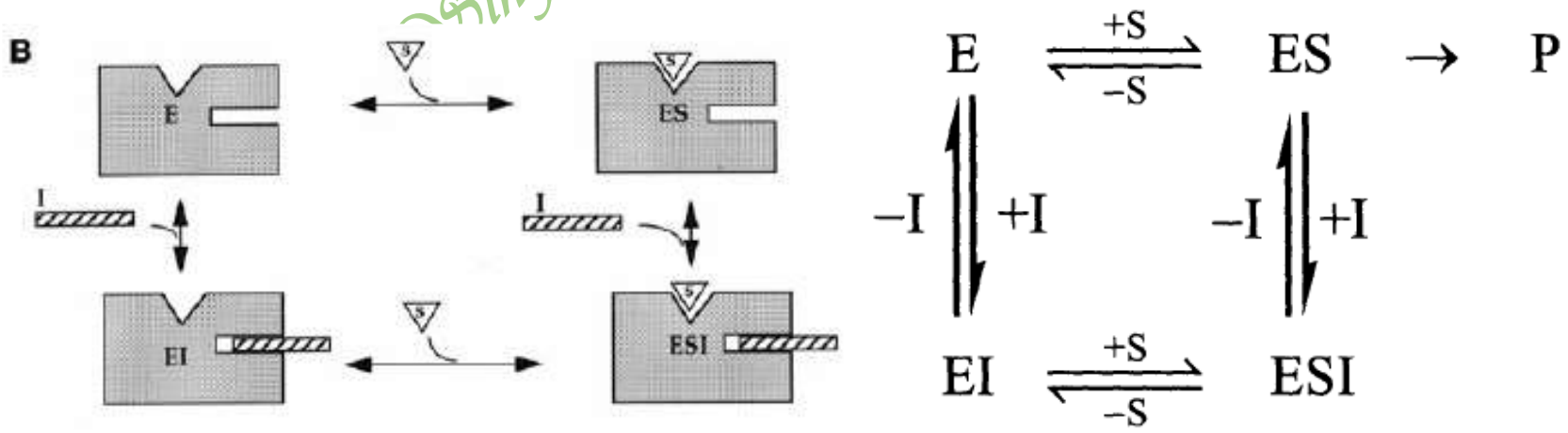
Non-competitive Inhibition

A non-competitive inhibitor can combine with an enzyme molecule to produce a **dead-end complex,** regardless of whether a substrate molecule is bound or not.

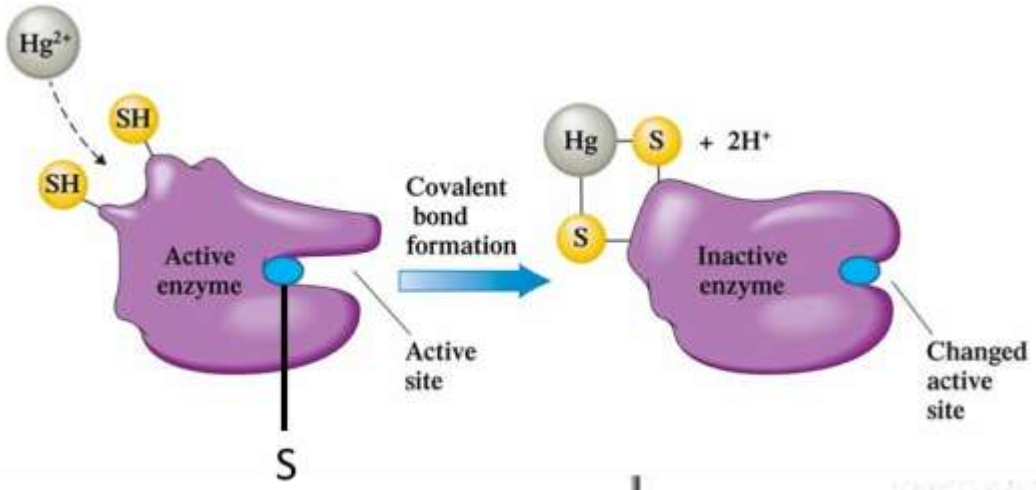
Hence the inhibitor must bind at a different site from the substrate.

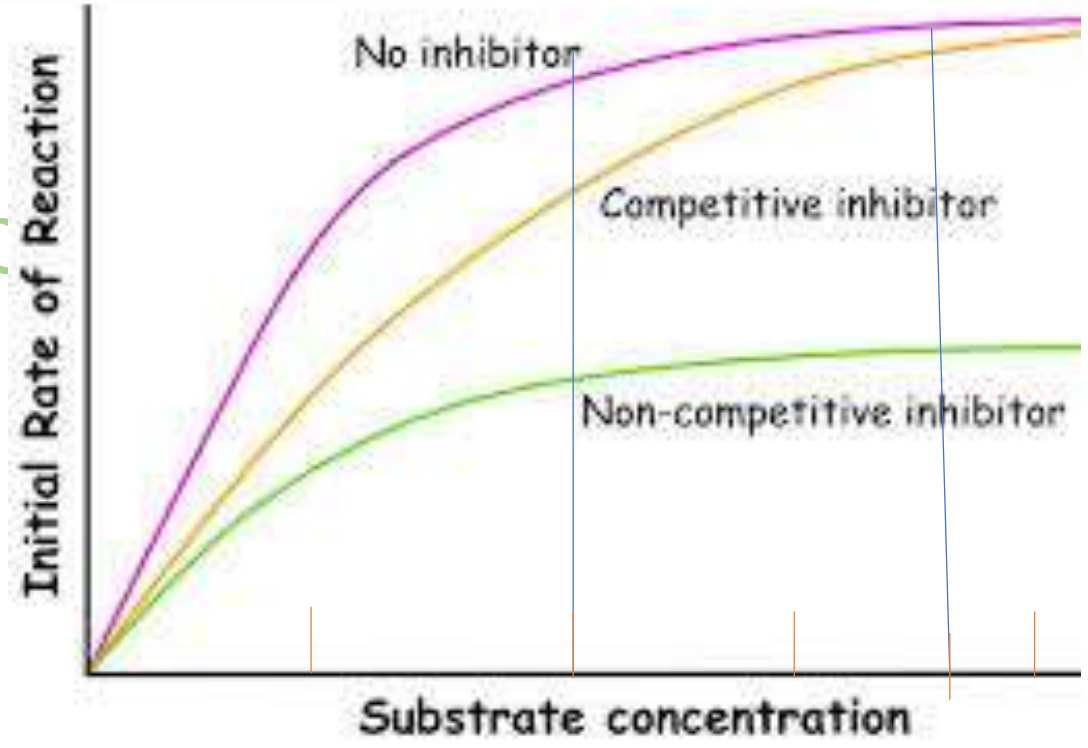
The inhibitor destroys the catalytic activity of the enzyme, either by binding to the catalytic site or as a result of a conformational change affecting the catalytic site, but does not affect substrate-binding .

The situation for a simple single-substrate reaction will be as follows:



Non-competitive Inhibition





Non-competitive Inhibition

- In the simplest possible model, **simple linear non-competitive inhibition,** the substrate does not affect inhibitor-binding.
- Under these conditions, the reactions

$$E + I \rightleftharpoons EI$$

$$\text{and} \qquad ES + I \rightleftharpoons ESI$$

have an identical dissociation constant *Ki* , again called the **inhibitor constant.**

- The total enzyme concentration is effectively reduced by the inhibitor, decreasing the value of *V*max but not altering *Km*, since neither inhibitor nor substrate affects the binding of the other.
- Let us once again derive an initial velocity equation

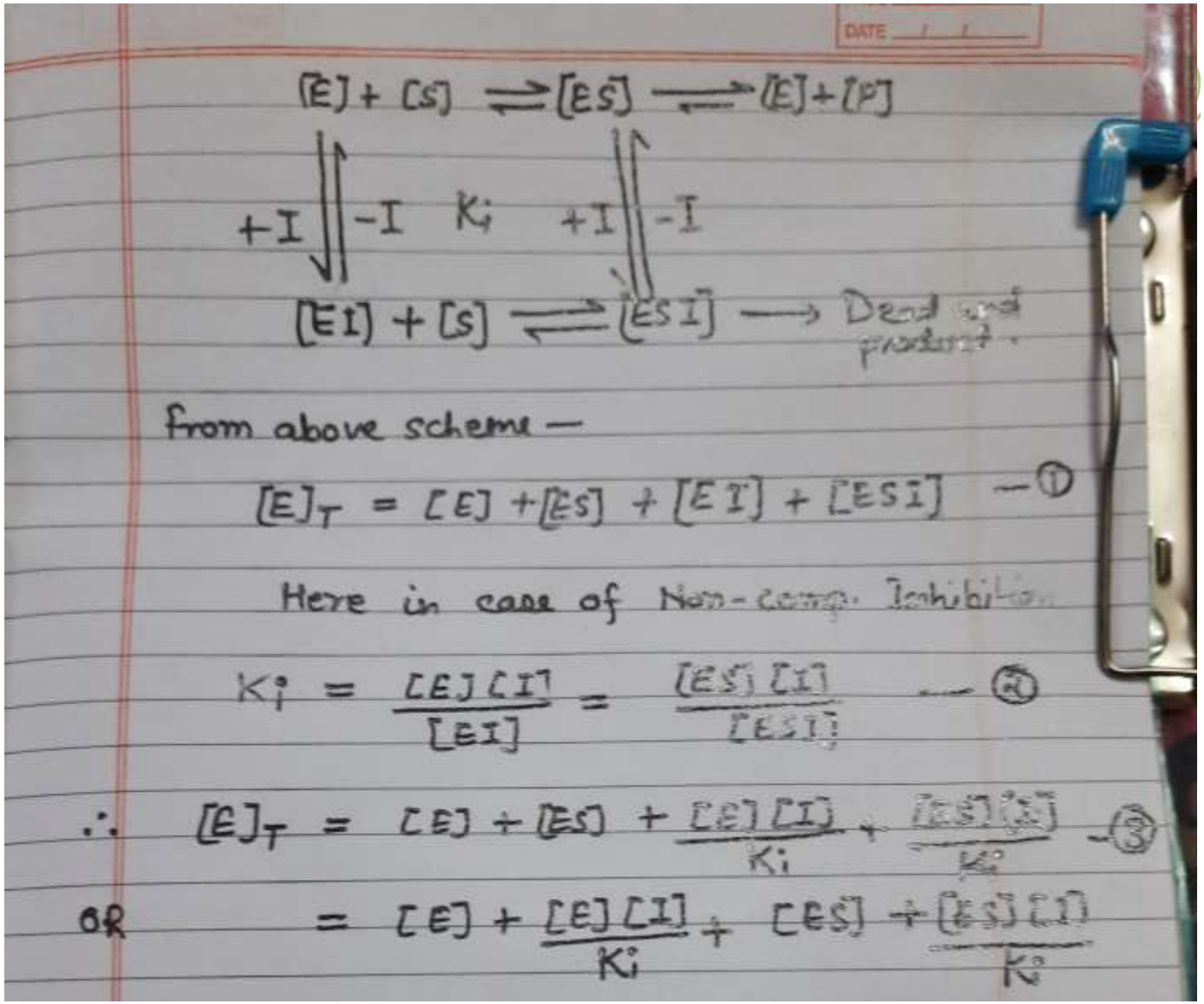
$$\text{so} \qquad [E][S]/[ES] = Km$$

- In the presence of a non-competitive inhibitor which will bind equally well to E or to ES,

i.e. where $K_i = [E][I]/[EI]$ OR $[ES][I]/[ESI]$:

$$[E]_T = [E] + [ES] + [EI] + [ESI]$$

Non-competitive Inhibition



Non-competitive Inhibition

OR $[E]_T = [E]\left(1 + \frac{[I]}{K_i}\right) + [ES]\left(1 + \frac{[I]}{K_i}\right)$ — (4)

OR $[ES]\left(1 + \frac{I}{K_i}\right) = [E_T] - [E]\left(1 + \frac{[I]}{K_i}\right)$.

OR $[ES]\left(1 + \frac{I}{K_i}\right) = [E_T] - \frac{K_m [ES]}{[S]}\left(1 + \frac{I}{K_i}\right)$ — (5)

OR $[ES]\left(1 + \frac{I}{K_i}\right) + \frac{K_m [ES]}{[S]}\left(1 + \frac{I}{K_i}\right) = [E_T]$ — (6)

OR $[ES]\left(1 + \frac{I}{K_i}\right)\left\{\left(1 + \frac{K_m}{[S]}\right)\right\} = [E]_T$ — (7)

Non-competitive Inhibition

OR $[ES]\left(1+\frac{I}{K_i}\right) = \frac{[E_T]}{\frac{[S]+K_m}{[S]}}$

$= \frac{[E]_T[S]}{K_m+[S]}$

OR $[ES] = \frac{[E]_T/\left(1+\frac{I}{K_i}\right)\cdot[S]}{K_m+[S]}$ — (8)

OR $\because [ES] \propto v_0$ & $[E]_T \propto V_{max}$

$\therefore v_0 = \frac{\frac{V_{max}}{(1+I/K_i)}\cdot[S]}{K_m+[S]}$ — (9)

Non-competitive Inhibition

$$[E_0] = [E] + [ES] + [EI] + [ESI]$$

$$= [E] + [ES] + \frac{[E][I]}{K_i} + \frac{[ES][I]}{K_i}$$

$$= ([E] + \{ES\})\left(1 + \frac{[I]}{K_i}\right)$$

$$\therefore [E] + [ES] = \frac{[E_0]}{\left(1 + \frac{[I]}{K_i}\right)}$$

$$\therefore [E] = \frac{[E_0]}{\left(1 + \frac{[I]}{K_i}\right)} - [ES]$$

Non-competitive Inhibition

$$v_0 = \frac{V_{max}}{\left(1+\frac{[I_0]}{K_i}\right)} \cdot \frac{[S_0]}{([S_0]+K_m)}$$

$$V'_{max} = \frac{V_{max}}{\left(1+\frac{[I_0]}{K_i}\right)}$$

Non-competitive Inhibition

This is of the form of the Michaelis-Menten equation, with Vmax being divided by a factor (1 + ([Io]/Ki)). Thus, for simple linear non-competitive inhibition, *Km* is unchanged and *V* max is altered so that:

$$V'_{max} = \frac{V_{max}}{\left(1+\frac{[I_0]}{K_i}\right)}, \quad \text{or} \quad \frac{1}{V'_{max}} = \frac{1}{V_{max}}\left(1+\frac{[I_0]}{K_i}\right)$$

where V'_{max} is the value of V_{max} in the presence of a concentration $[I_0]$ of noncompetitive inhibitor. It follows that *Ki* for such a system is the inhibitor concentration which halves the value of *V* max· The Lineweaver-Burk equation for simple linear non-competitive inhibition is:

$$\frac{1}{v_0} = \frac{K_m}{V'_{max}} \cdot \frac{1}{[S_0]} + \frac{1}{V_{max}}$$

and Lineweaver-Burk plots showing the effect of such inhibition are shown in

Non-competitive Inhibition

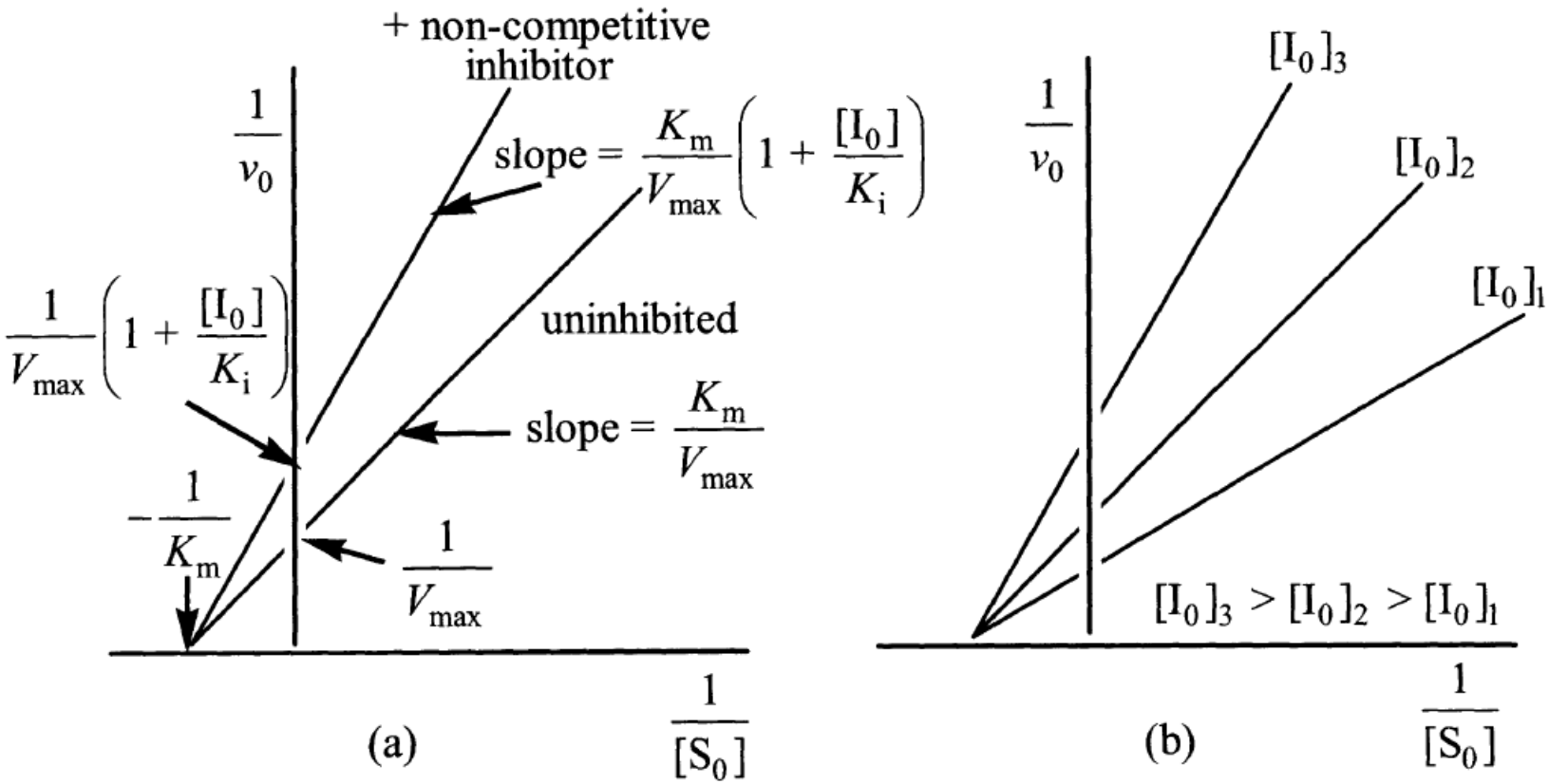


Fig. - (a) Lineweaver-Burk plot for simple linear non-competitive inhibition; (b) the same, showing plots for several inhibitor concentrations at fixed enzyme concentration.

TABLE 8.1 Examples of Competitive Enzyme Inhibitors

Substance	Target Enzyme	Process/Disease
Acetazolamide	Carbonic anhydrase	Glaucoma
N-Acetylglucosamine	Hexokinase	Glycolysis
Allopurinol	Xanthine oxidase	Gout
1-Amino-oxy-3-aminopropane	Ornithine decarboxylase	Cancer
o-Aminobenzoyl-Phe-Arg-Arg-Pro-Arg-*N*-(2,4-dinitrophenyl) ethylene diamine	Kallikrein-releasing enzyme	Hemostasis
Aminomethylbenzoic acid	Epithelial peptide transporter PepT1	
AppppA and AppppppA	Adenylate kinase (myokinase)	
5′-p(CH_2)ppA and 5′-p(CF_2)ppA	Most ATP-dependent enzymes	
5′-pp(CH_2)pA	Some ATP-dependent enzymes	
5′-p(NH)ppA	Most ATP-dependent enzymes	
[γ-S]-ATP	Most ATP-dependent enzymes (substrate for others)	
Caffeine	cAMP phosphodiesterase	
Captopril	Angiotensinogen-converting enzyme	Hypertension
Desulfo-Coenzyme A	Coenzyme A dependent enzymes	
Enalaprilate	Angiotensinogen-converting enzyme	Hypertension
Flutamide	Cytochrome P450 (CYP1B1)	Cancer
Gleevec	Bcr-Abl kinase	
Glyphosate	5-Enoylpyruvylshikimate-3-phosphate synthase	Weed control
5′-p(CH_2)ppG and 5′-p(CF_2)ppG	Most GTP-dependent enzymes	
5′-pp(CH_2)pG	Some GTP-dependent enzymes	
5′-p(NH)ppG	Most GTP-dependent enzymes	
[γ-S]-GTP	Most GTP-dependent enzymes (substrate for others)	
Hirudin	Thrombin	Hemostasis
Imatinib	Bcr-Abl kinase	Cancer
Indinavir	HIV polyprotein proteinase	HIV-AIDS
Levitra	cGMP phosphodiesterase (isozyme-5)	Erectile dysfunction
Lovostatin	Hydroxymethylglutaryl-CoA reductase	Hypercholesteremia
Lysine	Arginase	
Malonate	Succinate dehydrogenase	

IRREVERSIBLE INHIBITION

- An irreversible inhibitor binds to the active site of the enzyme by an irreversible reaction,

$$E + I \longrightarrow EI$$

and hence cannot subsequently dissociate from it.

- A covalent bond is usually formed between inhibitor and enzyme.
- The inhibitor may act by preventing substrate-binding or it may destroy some component of the catalytic site.
- Compounds which irreversibly denature the enzyme protein or cause non-specific inactivation of the active site are not usually regarded as irreversible inhibitors.
- An inhibitor which shows great affinity for the enzyme (dissociation constant in the order of 10^{-9} mol $l^{-1)}$ is regarded as irreversible.
- Unlike reversible inhibition, irreversible inhibition is progressive and will increase with time until either all the inhibitor or all the enzyme present has been used up in forming enzyme-inhibitor complex.
- Irreversible inhibitors can be used to map the active site, becoz they covalently bound to the enzyme active site and modify the functional groups, which can then be identified.

- Irreversible inhibitors effectively reduce the concentration of enzyme present.
- An inhibitor of initial concentration [Io] will reduce the concentration of active enzyme from an initial value of [Eo] to [Eo]-[Io]
- If a substrate is introduced after the reaction between inhibitor and enzyme has gone to completion, a system which obeys the Michaelis-Menten equation in the absence of inhibitor will still do so.
- **The value of K_m will be the same as for the uninhibited reaction, but V_{max} will be reduced (to V'_{max})**

In the absence of inhibitor, $V_{max} = k_{cat}[E_0]$

In the presence of inhibitor, $V'_{max} = k_{cat}([E_0] - [I_0])$

$$\therefore \frac{V'_{max}}{V_{max}} = \frac{[E_0]-[I_0]}{[E_0]}$$

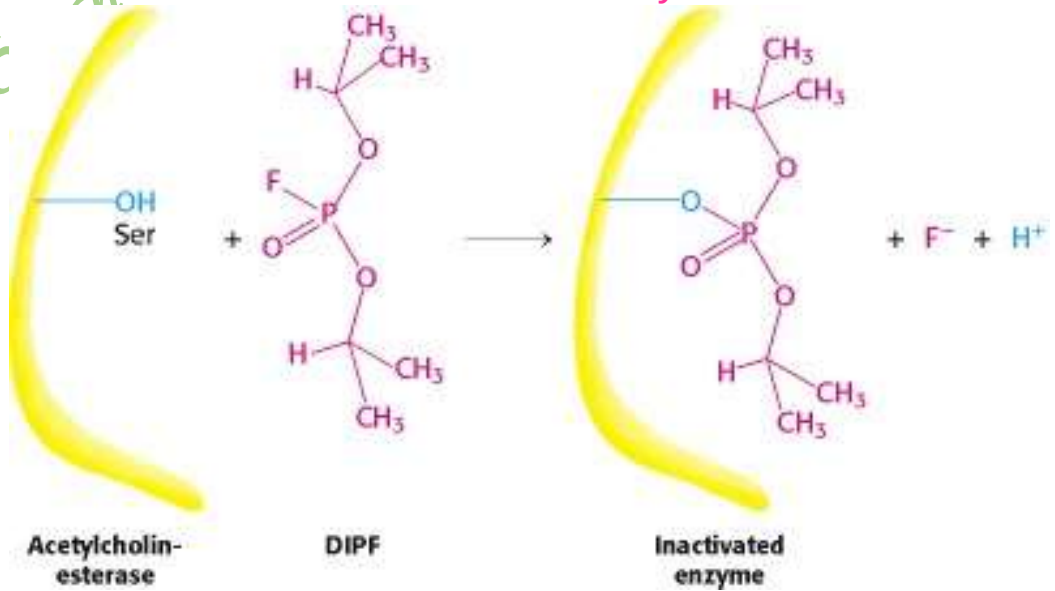
$$\therefore V'_{max} = V_{max}[E_0]\left(1-\frac{[I_0]}{[E_0]}\right)$$

Group-specific reagents

Irreversible inhibitors can be divided into three categories: group-specific reagents, substrate analogs, and suicide inhibitors.

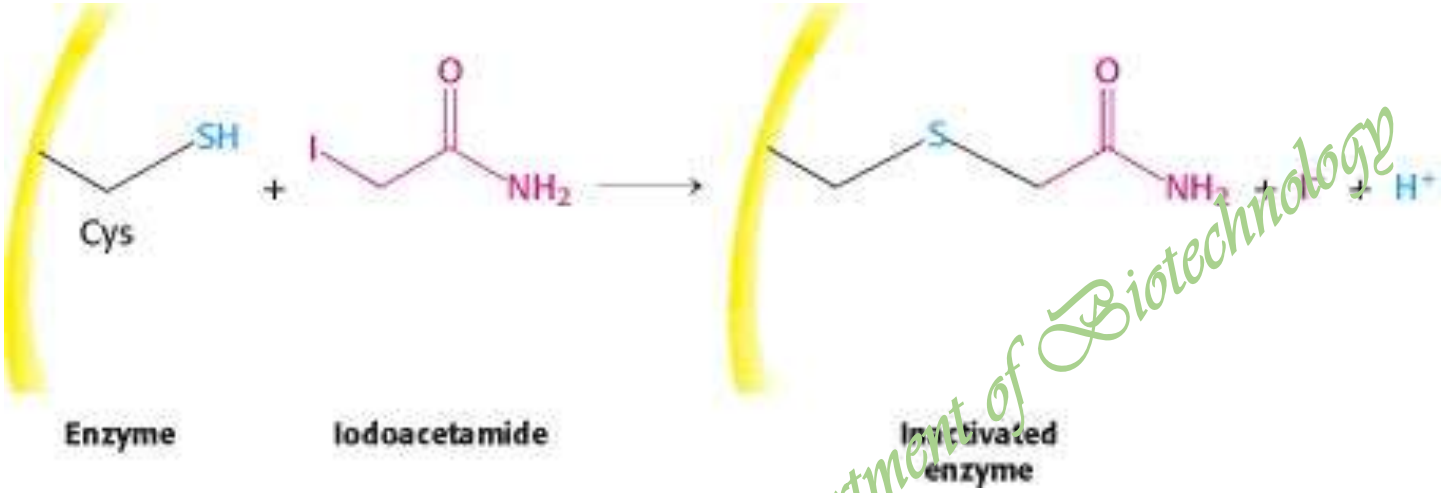
Group-specific reagents react with specific R groups of amino acids. Two examples of group-specific reagents are diisopropylphosphofluoridate and iodoacetamide.

- DIPF modifies only 1 of the 28 serine residues in the proteolytic enzyme chymotrypsin, implying that this serine residue is especially reactive.
- DIPF also revealed a reactive serine residue in acetylcholinesterase, an enzyme important in the transmission of nerve impulses
- Thus, DIPF and similar compounds that bind and inactivate acetylcholinesterase are potent nerve gases.



Enzyme Inhibition by Diisopropylphosphofluoridate (DIPF), a Group-Specific Reagent

Group-specific reagents



Enzyme Inactivation by Iodoacetamide, a Group-Specific Reagent.

Iodoacetamide can inactivate an enzyme by reacting with a critical cysteine residue.

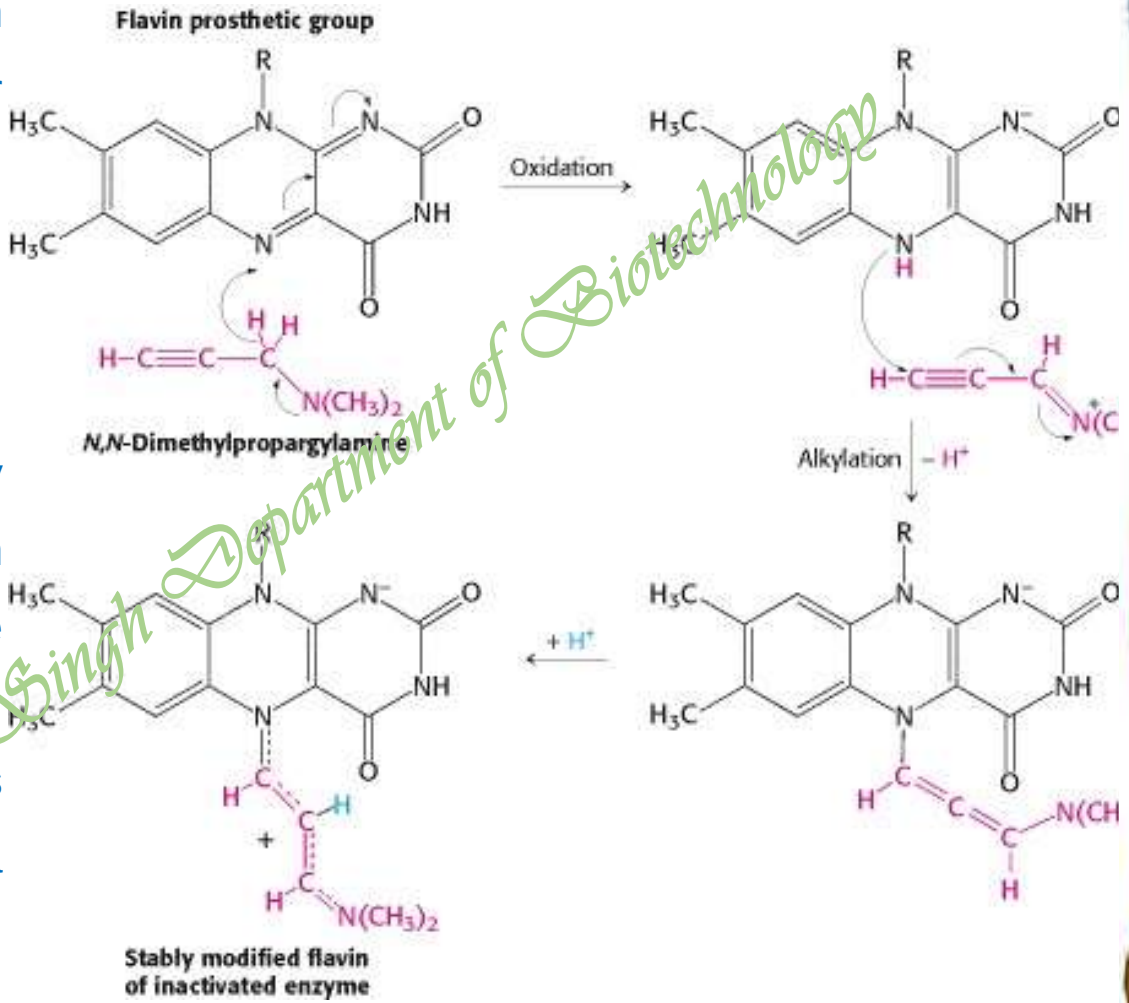
Suicide Inhibitors

- *Suicide inhibitors*, or *mechanism-based inhibitors* are modified substrates that provide the most specific means to modify an enzyme active site.
- The inhibitor binds to the enzyme as a substrate and is initially processed by the normal catalytic mechanism.
- The mechanism of catalysis then generates a chemically reactive intermediate that inactivates the enzyme through covalent modification.
- ***N,N*-dimethylpropargylamine is a suicide inhibitor for Monamine Oxidase**
- Monoamine oxidase deaminates neurotransmitters such as dopamine and serotonin, lowering their levels in the brain.
- Monoamine oxidase (MAO) oxidizes the *N*,*N*-dimethylpropargylamine, which in turn inactivates the enzyme by covalently modifying its flavin prosthetic group by alkylating N-5 .
- Parkinson disease is associated with low levels of dopamine, and depression is associated with low levels of serotonin. The drug (-)deprenyl, which is used to treat Parkinson disease and depression, is a suicide inhibitor of monoamine oxidase.

Suicide Inhibitors

Monoamine oxidase, an enzyme important for neurotransmitter synthesis, requires the cofactor FAD (flavin adenine dinucleotide). *N*,*N*-Dimethylpropargylamine inhibits monoamine oxidase by covalently modifying the flavin prosthetic group only after the inhibitor is first oxidized.

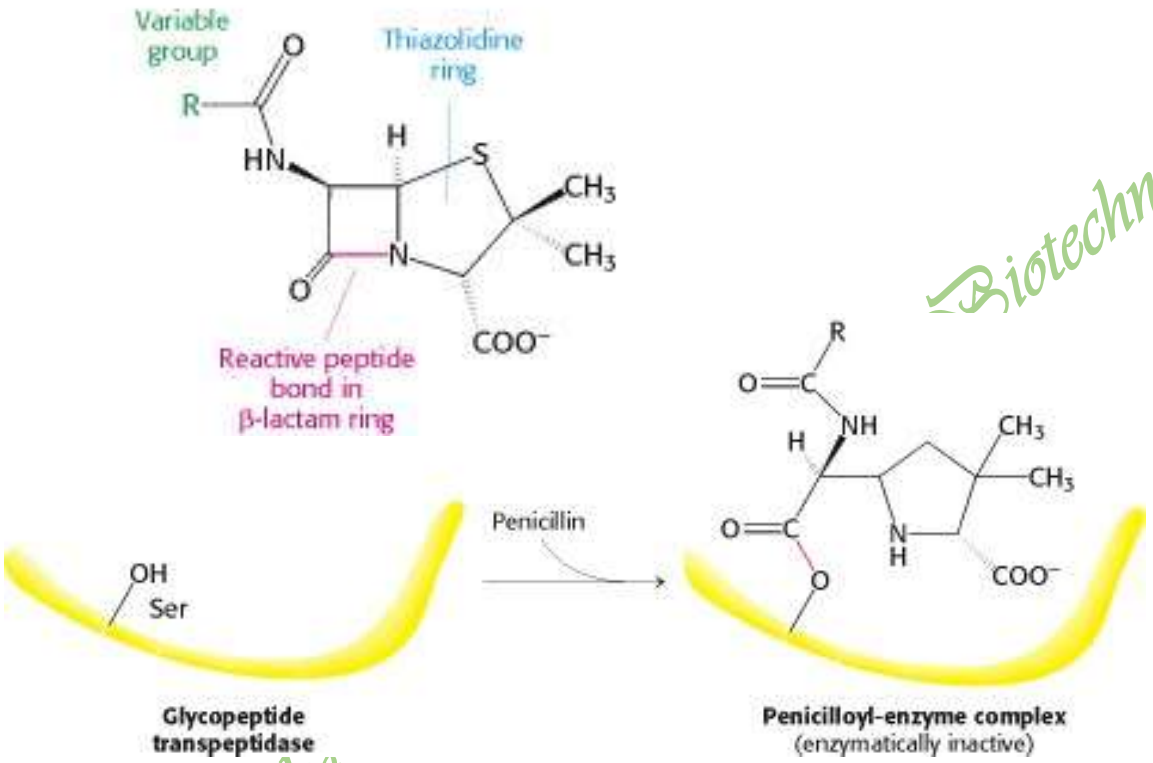
The N-5 flavin adduct is stabilized by the addition of a proton.



Selegiline, also known as **L-deprenyl** OR **Eldepryl** is a selective, irreversible inhibitor of Type B monoamine oxidase that is used for the treatment of newly diagnosed patients with PARKINSON DISEASE, and for the treatment of depressive disorders.

Penicillin Is An Effective *Suicide Inhibitors* Of The Transpeptidase

- Penicillin inhibits the cross-linking transpeptidase by the Trojan horse stratagem.
- The transpeptidase normally forms an *acyl intermediate* with the penultimate D-alanine residue of the D-Ala-D-Ala peptide.
- This covalent acyl-enzyme intermediate then reacts with the amino group of the terminal glycine in another peptide to form the cross-link.
- Penicillin is welcomed into the active site of the transpeptidase because it mimics the D-Ala-D-Ala moiety of the normal substrate.
- Bound penicillin then forms a covalent bond with a serine residue at the active site of the enzyme. *This penicilloyl-enzyme does not react further. Hence, the transpeptidase is irreversibly inhibited and cell-wall synthesis cannot take place*.
- The highly strained, four-membered β-lactam ring of penicillin makes it especially reactive. On binding to the transpeptidase, the serine residue at the active site attacks the carbonyl carbon atom of the lactam ring to form the penicilloyl-serine derivative.
- Because the peptidase participates in its own inactivation, penicillin acts as a suicide inhibitor.



Formation of a Penicilloyl-Enzyme Complex

Penicillin reacts with the transpeptidase to form an inactive complex, which is indefinitely stable.